STUDY TITLE

Summary of Companion Animal Safety Data For
Effipro ™ Plus Topical Solution for Cats
(Fipronil 9.5% and Pyriproxyfen 11.5% End Use Product)

DATA REQUIREMENTS

Companion Animal Safety (OPPTS 870.7200)

PERFORMING LABORATORY

ISAVET BIOSCIENCES
8, Allée Romain Rolland
93390 Clichy sous Bois
FRANCE

STUDY NO.

Virbac 104.15-1

STUDY COMPLETION DATE

December 1, 2011

PAGE 1 OF 27

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

No claim of is made for any information contained in this report on the basis of it falling within the scope of FIFRA Sec. 10(d)(A)(B) or (C).

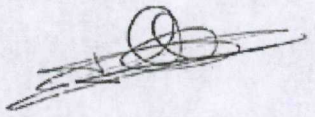
Company: Virbac AH, Inc.
PO Box 162059
Fort Worth TX 76161

Company Agent: Craig Lahr Date: Dec-5-2011
VIRBAC, SA

GOOD LABORATORY PRACTICE STATEMENT

This document is a compilation document and is not subject to the requirements of 40 CFR Part 160.

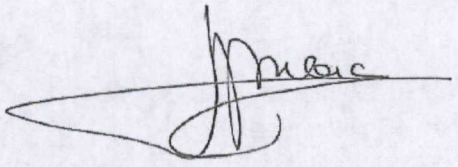
REPORT AUTHOR:



Dec-1-2011

Isabelle Villard, DVM — Date
Isavet Biosciences

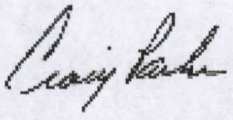
SPONSOR:



Dec-3-2011

Jean-Pascal Marc, DVM. — Date
Group Product Innovation Director
Virbac SA.

SUBMITTER:



Dec-5-2011

Craig Parks, MS, DVM. — Date
Vice President, R&D
Virbac AH, Inc.

TABLE OF CONTENTS

Introduction

Effipro™ Plus Topical Solution for Cats is an end-use product based upon the common dose rate and dose volume for the fipronil (only) spot-on products. Effipro™ Plus Topical Solution for Cats is then enhanced with pyriproxyfen to provide a 6-week claim for flea eggs and larvae.

As demonstrated in the table below, Effipro™ Plus Topical Solution for Cats (9.5% fipronil) has the same dose size in terms of mg of fipronil as the fipronil (only) spot-on registered products, and in particular to the registered Frontline® Top Spot® For Cats (EPA Reg. No. 65331–2), and Virbac's registered Effipro® Topical Solution for Cats (EPA Reg. No. 2382-186).

Frontline® Top Spot® for Cats EPA Reg. No. 65331-2		Effipro® Topical Solution for Cats EPA Reg. No. 2382-186		Effipro™ Plus Topical Solution for Cats EPA Reg. No. 2382-NEW	
Volume	mg Fipronil	Volume	mg Fipronil	Volume	mg Fipronil
0.50 ml	50 mg	0.50 ml	50 mg	0.50 ml	50 mg + 60 mg pyriproxyfen

As Effipro™ Plus Topical Solution for Cats has the exact same doses in terms of mg fipronil as the fipronil-only spot-on products, the previously conducted and Agency accepted fipronil companion animal safety studies are appropriate to support the companion animal safety for the fipronil portion of Effipro™ Plus Topical Solution for Cats. Moreover, in addition to a number of laboratory safety studies with cats and kittens, the Frontline® Top Spot® For Cats fipronil product (registered in 1996) has been very widely used and has been shown to be safe in the most difficult test – when it is used widely under all conditions on millions and millions of cats and kittens for more than15 years.

With regards to the pyriproxyfen component of Effipro™ Plus Topical Solution for Cats, the use rate in terms of mg per cat is in the range of pyriproxyfen use rates used in very similar spot-on products registered with the EPA.

Indeed, pyriproxyfen is very often used alone or in combination to active ingredients having a flea adulticide activity in veterinary spot-on products. In these products, pyriproxyfen is included at various dose ranges from approximately 1 to 4 mg pyriproxyfen per cat with Advantage II Kitten (EPA Reg. No. 11556 – 150) and Advantage II Large Cat (EPA Reg. No. 11556 - 152), and up to 80 mg per cat with Farnam F274 (EPA Reg. No. 270-308). Virbac currently has registered a pyriproxyfen spot-on product for cats (EPA Reg. No. 2382-173). The product is a 10% pyriproxyfen spot on for which each 0.6 ml applicator delivers a total dose of 60 mg of pyriproxyfen per cat, as it is the case for each applicator of Effipro™ Plus Topical Solution for Cats. The volume for each dose of Effipro™ Plus Topical Solution for Cats is only 0.5 ml, but this formulation contains more pyriproxyfen and consequently delivers the same total amount of pyriproxyfen to the cat (60 mg).

	Effipro Plus ™ Topical Solution for Cats EPA Reg. No. 2382-NEW		Pyriproxyfen 10% Spot On for Cats EPA Reg. No. 2382-173	
Volume	mg Fipronil	mg Pyriproxyfen	Volume	mg Pyriproxyfen
0.50 ml	50 mg	60 mg	0.60 ml	60 mg

Therefore, the proposed pyriproxyfen dose rate to be used in Effipro™ Plus Topical Solution for Cats falls within a dose rate range of the already approved products, and corresponds exactly with the dose rate of EPA Reg. No. 2382-173. Therefore, the previously conducted and Agency accepted pyriproxyfen companion animal safety studies on the many various spot on products delivering at least 60 mg of pyriproxyfen per cat are appropriate to support the companion animal safety for the pyriproxyfen component of Effipro ™ Plus Topical Solution For Cats.

I. Data citation

Fipronil data:

To support the safety of Effipro™ Plus Topical Solution For Cats, the following fipronil cat safety data are being cited. Please note, some of the early cat safety studies were done with the 0.25% spray product (EPA Reg. No. 65331 – 1), and then these data were later bridged to support the Frontline® Top Spot® For Cats (EPA Reg. No. 65331-2). Consequently, both the spray product and the spot on cat safety studies support the safety of the fipronil component of Effipro™ Plus Topical Solution For Cats, and these studies are cited in the table below.

Guideline	Study	MRID #
870.7200	Cat safety	43272401
870.7200	Cat safety	43444904
870.7200	Cat safety	43863801
870.7200	Cat safety	43121112
870.7200	Cat safety	44942009

These data and the more than 15 years on the market for Frontline® Top Spot® For Cats have demonstrated the safety of fipronil on cats and kittens as young as 8 weeks of age at the 50 mg per cat/kitten dose. As this is the same fipronil dose for the proposed Effipro™ Plus Topical Solution For Cats, this product should also be considered safe on cats and kittens as young as 8 weeks of age.

Pyriproxyfen data:

To support the cat and kitten safety of Effipro™ Plus Topical Solution For Cats, the following pyriproxyfen safety data already accepted by the EPA for Virbac's Pyriproxyfen 10% Spot On for Cats, EPA Reg. No. 2382-173, are being cited.

MRID #	Date of submission	Product	Study Title
44850103	04/21/1999	10% Pyriproxyfen spot on	Tolerance Study by Cutaneous Application (Spot On) in cats: Pyriproxyfen 10% Spot On For Cats : Lab Project Number: 636.29 (Virbac study number F-636.29/0007)

These data were reviewed by the Agency (October 25, 1999 Dr. Byron Backus memo see appendix) and accepted for the registration of the Pyriproxyfen 10% Spot On for Cats product on cats 3 months of age or older. As noted in the Agency review of the study, the study was conducted before publication of the OPPTS 870.7200 guideline companion animal safety studies.

The proposed age limit for the Effipro™ Plus Topical Solution for Cats is 8 weeks as per the age limit on Effipro and Frontline.

Although the study cited above did not use kittens 8 weeks of age, pyriproxyfen has very low toxicity to mammals. Moreover, many years ago (again before the publication of the OPPTS 870.7200 guideline), Virbac conducted a study with this pyriproxyfen formulation using one month old kittens. The report on this study provides additional supplemental safety data to support the 8-week kitten claim on the new product. This study which has never been submitted to the Agency, was also conducted before the publication of the OPPTS 870.7200 guideline, is summarized below, and the final study report is being concurrently submitted.

II. Summary of 10% Pyriproxyfen Spot On Safety Studies

MRID No. 44850103 (3 month kittens)

See DER attached as appendix.

Virbac study No. 636.29 40006 (1 month kittens)

See final study report being concurrently submitted.

STUDY TYPE: Companion Animal Safety/one month old kittens – non-guideline

TEST MATERIAL: Pyriproxyfen

STUDY Number: 15658TSF

TESTING FACILITY: Centre International de Toxicologie (C.I.T.)

SPONSOR: VIRBAC SA., 13ème rue LID, BP27, F-06511 Carros Cedex, France.

EXECUTIVE SUMMARY:
A total of 21 cats (9 males and 12 females) were randomly allocated to two treated and one control group. Initially, 18 animals (9 males and 9 females) were assigned to the treatment groups. Three females (two from the control group and one from the high dose group) died or

were euthanized between days 10 and 15. These 3 animals were replaced by three additional animals (supplementary animals). During the study, the cats were maintained with their mothers.
The animals were one month-old at the time of treatment (mean bodyweight: 394g for the males and 406g for the females) while the supplementary animals were 1.5 month-old at the time of treatment (mean bodyweight: 537g).
Animals in the control group (6 animals + 2 supplementary animals) received 1.8 ml of excipient by cutaneous application.
Animals in the low dose group (6 animals) received 0.6 ml of test article (10% pyriproxyfen spot-on) by cutaneous application.
Animals in the high dose group (6 animals + 1 supplementary animal) received 1.8 ml of test article by cutaneous application. Products were applied on the skin at the base of the skull.
Each animal was given the test or control substance once a day for three consecutive days during two consecutive treatment periods (days 1, 2 and 3 and then days 19, 20 and 21). The supplementary animals received products only during one treatment period (days 1, 2 and 3).
After the last treatment was received, all animals were observed for 16 days.
The animals were monitored for mortality and morbidity, clinical signs of toxicity, bodyweight, electrocardiographic examinations, ophthalmological examinations, haematology, blood chemistry during the study and sacrificed on day 38.
No death considered to be related to treatment with the test substance or vehicle occurred during the study. The deaths of the three kittens were due to unspecific decline of clinical condition, possibly related to rejection by the mother.
No clinical signs indicating of toxicity were seen in the mothers and surviving kittens.
Mean bodyweights were similar between groups throughout the study.
No treatment related changes were reported at the electrocardiographic and ophthalmological examinations, in the haematological parameters or in the blood chemistry parameters.
At necropsy, macroscopic and microscopic findings were found in the kidneys of animals that died prematurely. These findings were probably related to the poor physical condition and dehydration of the cats.
No macroscopic post-mortem changes were observed at necropsy in the animals that survived until the end of the study.

This study demonstrated that a 10% pyriproxyfen solution at dose levels of 0.6 and 1.8 ml/animals for two periods of three days is well-tolerated when applied on the skin of one-month old kittens.

III. Conclusion

Many previous safety studies and more than 15 years of use have demonstrated the safety of fipronil and pyriproxyfen separately on cats and kittens at the dose levels proposed for Effipro™ Plus Topical Solution For Cats. Moreover, the safety studies conducted with a pyriproxyfen spot-on in young and very young kittens confirmed the tolerance of pyriproxyfen at the same dose rate even at exaggerated doses.

These safety data support the registration of Effipro™ Plus Topical Solution For Cats.

Appendix: DER for MRID 448501-03 (October 25th 1999)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

October 25, 1999

MEMORANDUM

EPA File Symbol: 2382-RTG Pyriproxyfen 10% Spot On For Cats
DP Barcode: D257141
Case No: 065865
PC Code: 129032 Pyriproxyfen, Nylar

From: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505C)

Byron T. Backus
10/25/99

To: Joseph Tavano/Arnold Layne PM 03
Insecticide Branch
Registration Division (7505C)

Registrant: Virbac Inc.

ACTION REQUESTED: "Review label and data for Domestic [Companion] Animal Safety. MRID 448501-03."

BACKGROUND: According to a cover letter from the registrant, this is an application for the registration of product containing 10% Pyriproxyfen. The registrant is requesting a waiver from the requirement of conducting an acute inhalation toxicity study as "the volume of the unit dose, its packaging and method of application are unlikely to ever result in the generation of inhalable particles." According to the proposed label "Nylar IGR sterilizes fleas and (kills) flea eggs for 3 months." The label also states that the product, as packaged, consists of four pipettes, each containing 0.6 g of product. This application includes a Companion Animal Safety Study (MRID 44850103) which was conducted on 3-month old kittens. This study was conducted at Centre International de Toxicologie (C.I.T.) Miserey, 27005 Evreux, France. This study was

reviewed at Oak Ridge, and the resulting DER was secondarily reviewed and modified by TRB staff, before being sent to HED. There was a meeting on October 13, 1999 between representatives from TRB and HED; subsequently, TRB received a memorandum (dated October 19, 1999, copy attached) from Virginia Dobozy, V.M.D., providing an additional review of the DER.

COMMENTS AND RECOMMENDATIONS:

Based on the packaging of the product (capsules or pipettes, each containing 0.6 g of a formulation with 10% Pyrproxyfen as sole active), TRB recommends for issuance of a waiver of the acute inhalation LC50 study on this formulation.

HED has stated that "given dosing at less than 5x the label recommended treatment and the use of only three animals/sex/group, RD should reconsider whether this study should be classified as acceptable." In the meeting on October 13, HED expressed concerns regarding whether the dosing scheduling (1X, 2X and 3X applied on days 1, 2, 3, for cumulative dosages of 3X, 6X and 9X) did, in fact, establish a 5X safety factor; however, the use of three animals/sex/group was not discussed at that time. TRB notes that the study submitted by Virbac was initiated on July 1, 1997 and completed on February 2, 1998. The OPPTS Harmonized Test Guidelines (including OPPTS 870.7200, Companion animal safety, specifying the use of at least six animals per sex) were published in August 1998.

With respect to the companion animal safety study, the following is the executive summary from the DER for MRID 44850103. This executive summary has been revised based on the comments received from HED:

In a companion animal safety study (MRID 44850103), a formulation containing 10% pyriproxyfen as sole active was topically applied to groups of three male and three female 3-month old kittens for two periods of three consecutive days (days 1, 2, and 3 and then days 19, 20 and 21) at 1X (0.6 mL/treatment), 2X (1.2 mL/treatment), and 3X (1.8 mL/treatment) label-indicated dosing levels/day (3X, 6X and 9X over each 3-day dosing period). Controls were treated with 1.8 mL of excipient. While the number of animals/sex/dosage group is below the six specified in OPPTS 870.7200, the study was initiated on July 1, 1997, and completed on February 2, 1998, before the publication (August 1998) of the OPPTS Harmonized Test Guidelines. Test animals were observed for toxicologic effects once per hour for 4 hr after each application and daily during the entire 36-day study. Food consumption was monitored daily. Body weights were recorded twice before treatment began and once a week thereafter. Blood samples for hematological and biochemical analyses were taken before treatment began and 24 hr after the last dose of each treatment period. Blood samples for cholesterol analysis were taken on days 10 and 28. Urinalysis, as well as electrocardiographic and ophthalmological examinations were performed before treatment and on days 18 and 36. All animals were sacrificed at the end of the study,

with post-mortem macroscopic examinations conducted on all. The skin application site was examined microscopically. Sacrifice and necropsy are not required by the Companion Animal Safety Study Guidelines, although as previously noted this study was conducted before the August 1998 publication of the OPPTS Harmonized Test Guidelines.

Although one animal died during the study, this death was not considered to be treatment-related. Microscopic examination did not reveal any treatment-related effects on the skin. Upon necropsy one female from the low-dose group showed a thickened urinary bladder with reddish foci, not considered to be treatment-related because similar effects were not seen in any of the animals in the higher dose groups. Food consumption and body weights were not affected by treatment. Moderate to markedly lower total cholesterol values (-24 to -52%) occurred at all sampling times in one male cat in the 1.2 mL/treatment group and in one female in the 1.8 mL/treatment group. These changes were not considered to be treatment-related because no trend was observed in females at the intermediate dose or in males at the highest dose. There were no other changes in clinical chemistry or hematology that could be attributed to treatment. Electrocardiographic and ophthalmological examinations did not reveal any abnormalities in the treated animals.

Although no single daily application of pyriproxyfen exceeded a 3X dosing level, a cumulative 6X exposure was achieved by the second day and a cumulative 9X exposure by the third day. The product was applied again to the same test animals 15-17 days after the first treatment with no adverse effects. Product labeling and application instructions state that it should be reapplied every three months. The label includes the statement: "Do not treat kittens less than one month." However, the kittens used in this study were three months old, and the statement should therefore be revised to stated: "Do not treat kittens less than three months." While there are questions as to whether or not the dosage schedule really demonstrated a 5X margin of safety, it is noted that the proposed product is packaged in pipettes, and would be dispensed in measured amounts of 0.6 g/application. As long as this product is packaged in this manner, this study is Acceptable to support the proposed use on cats over the age of 3 months. It is also noted that the available data indicate that the active ingredient in this formulation (Pyriproxyfen) has been demonstrated to have low toxicity to mammalian species.

DATA EVALUATION REPORT

PYRIPROXYFEN 10% SPOT ON FOR CATS

STUDY TYPE: Companion Animal Safety – Cat (OPPTS 870.7200)
MRID 44850103

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

Primary Reviewer:
Dennis M. Opresko, Ph.D. Signature: _____
Date: _____

Secondary Reviewers:
Cheryl B. Bast, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Eric Lewis, M.S. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Managed by Lockheed Martin Energy Research Corp., for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

EPA Reviewer:Byron T. Backus, Ph.D. _____ Date: _____
Technical Review Branch (7505C)
EPA Work Assignment Manager: John Redden, M.S. _____ Date: _____
Registration Division (7505C)

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety/3-Month Old Kittens [OPPTS 870.7200]

EPA I.D. NUMBERS: DP BARCODE: D 257141; MRID NUMBER: 44850103

TEST MATERIAL: Pyriproxyfen

STUDY NUMBER: 15645 TSF (Sponsor's reference: 636.29/0007)

TESTING FACILITY: Centre International de Toxicologie (C.I.T.)

SPONSOR: VIRBAC INC.

TITLE OF REPORT: Tolerance Study by Cutaneous Application (Spot On) in Cats

AUTHOR: C. Fabreguettes, Ph.D.

REPORT ISSUED: February 2, 1998

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 44850103), a formulation containing 10% pyriproxyfen as sole active was topically applied to groups of three male and three female 3-month old kittens for two periods of three consecutive days (days 1, 2, and 3 and then days 19, 20 and 21) at 1X (0.6 mL/treatment), 2X (1.2 mL/treatment), and 3X (1.8 mL/treatment) label-indicated dosing levels/day (3X, 6X and 9X over each 3-day dosing period). Controls were treated with 1.8 mL of excipient. While the number of animals/sex/dosage is below the six specified in OPPTS 870.7200, the study was initiated on July 1, 1997, and completed on February 2, 1998, before the publication (August 1998) of the OPPTS Harmonized Test Guidelines. Test animals were observed for toxicologic effects once per hour for 4 hr after each application and daily during the entire 36-day study. Food consumption was monitored daily. Body weights were recorded twice before treatment began and once a week thereafter. Blood samples for hematological and biochemical analyses were taken before treatment began and 24 hr after the last dose of each treatment period. Blood samples for cholesterol analysis were taken on days 10 and 28. Urinalysis, as well as electrocardiographic and ophthalmological examinations were performed before treatment and on days 18 and 36. All animals were sacrificed at the end of the study, with post-mortem macroscopic examinations conducted on all. The skin application site

was examined microscopically. Sacrifice and necropsy are not required by the Companion Animal Safety Study Guidelines, although as previously noted this study was conducted before the August 1998 publication of the OPPTS Harmonized Guidelines.

Although one animal died during the study, this death was not considered to be treatment-related. Microscopic examination did not reveal any treatment-related effects on the skin. Upon necropsy one female from the low-dose group showed a thickened urinary bladder with reddish foci, not considered to be treatment-related because similar effects were not seen in any of the animals in the higher dose groups. Food consumption and body weights were not affected by treatment. Moderate to markedly lower total cholesterol values (-24 to -52%) occurred at all sampling times in one male cat in the 1.2 mL/treatment group and in one female in the 1.8 mL/treatment group. These changes were not considered to be treatment-related because no trend was observed in females at the intermediate dose or in males at the highest dose. There were no other changes in clinical chemistry or hematology that could be attributed to treatment. Electrocardiographic and ophthalmological examinations did not reveal any abnormalities in the treated animals.

Although no single daily application of pyriproxyfen exceeded a 3X dosing level, a cumulative 6X exposure was achieved by the second day and a cumulative 9X exposure by the third day. The product was applied again to the same test animals 15-17 days after the first treatment with no adverse effects. Product labeling and application instructions state that it should be reapplied every three months. The label includes the statement: "Do not treat kittens less than one month." However, the kittens used in this study were three months old, and the statement should therefore be revised to stated: "Do not treat kittens less than three months." While there are questions as to whether or not the dsoage schedule really demonstrated a 5X margin of safety, it is noted that the proposed product is packaged in pipettes, and would be dispensed in measured amounts of 0.6 g/application. As long as this product is packaged in this manner, this study is Acceptable to support the proposed use on cats over the age of 3 months. It is also noted that the available data indicate that the active ingredient in this formulation (Pyriproxyfen) has been demonstrated to have low toxicity to mammalian species.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Statements were present.

I. MATERIALS

A. Test material: Pyriproxyfen 10% Spot On For Cats
Description: Colorless liquid
Lot/Batch No.: MS-7134
Active Ingredients: Pyriproxyfen (Nylar) 10%
Storage Conditions: Stored at room temperature away from light

B. Administration: topical

C. Vehicle and/or control

Vehicle: Not reported
Control: "excipient 636.29" Described as a colorless liquid.

D. Test animals

Species: Cat
Breed: ICO:Fec Eur (Tif)
Age and weight at study initiation: about 3 months old; mean body weight 1.1 kg (males 1.0-1.4 kg; females 1.0-1.1 kg).
Source: Iffa Crédo, l'Arbresle, France
Housing: Individually in stainless steel cages
Diet: Cat pellets (40 g/day), U.A.R. (Batch No. 024)
Water: Tapwater, *ad libitum*
Environmental conditions:
Temperature: 20° ± 5°C
Humidity: 50 ± 20%
Acclimation period: 13 days

II. STUDY DESIGN

A. In life dates

Start: August 19-21, 1997; end: September 26, 1997

B. Animal assignment/ Dosage and Administration

Cats "were allocated to the groups according to a computerised body weighyt stratification procedure, so that the average body weight of each group was similar" (Table 1). Groups of 3 cats/sex were topically treated with excipient (control) or with 1X, 2X, or 3X doses of the test material on Days 1, 2, 3, 19, 20, and 21. The last treatment was followed by a 15-day observation period. The animals were sacrificed on day 39. The test substance and control were applied to the skin at the dorsal base of the skull after parting of the hair.

Table 1. Experimental Design

Group	No. of animals		Treatment	Number of applications
	Male	Female		
1	3	3	Excipient (1.8 mL)	6

2	3	3	Test material (0.6 mL)	6
3	3	3	Test material (1.2 mL)	6
4	3	3	Test material (1.8 mL)	6

Data taken from pp. 10 and 13, MRID 44850103.

C. Dose selection rationale

The rationale for dose levels was to establish the margin of safety and potential dermal and systemic toxicity of 3X, 6X, and 9X the recommended topical application.

D. Experimental design

Following a 13-day pretest period, the animals were dosed topically on days 1, 2 and 3. The same animals were dosed again on days 19, 20 and 21. The animals were observed for an additional 15 days, and sacrificed on day 39. Animals were checked for clinical signs once per hour for 4 hr after each treatment and once per day on non-treatment days. The animals were observed for morbidity and mortality twice per day. Body weights were recorded before treatment and once per week until the end of the study. Food and water consumption was calculated daily. Electrocardiography and ophthalmological tests were performed before treatment and on days 18 and 36.

E. Pathological parameters

Blood samples were obtained from the cephalic or jugular vein once before the start of treatment and on days 4 and 22. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count	X	Reticulocyte count
X	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)*		
	(Activated partial thromboplastin time)*		
	Erythrocyte morphology		

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*		Globulin*
X	Sodium*	X	Glucose*
		X	Total and direct bilirubin*
	ENZYMES	X	Total serum protein* (TP)
X	Alkaline phosphatase(ALK)*		
	Cholinesterase(ChE)	X	Triglycerides
	Creatine kinase		Serum protein electrophoresis
	Lactic acid dehydrogenase(LDH)	X	Albumin/globulin ratio
X	Serum alanine amino- transferase (also		
X	SGPT)*		
	Serum aspartate amino- transferase(also SGOT)*		
	Gamma glutamyl transferase(GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. Statistics

Descriptive statistics and analysis of variance (Bartlett's, Fisher's, Dunn's, Student's or Mann-Whitney's tests) were employed

G. Disposition of animals

The animals were sacrificed on day 39.

H. Compliance

Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Statements were present.

III. RESULTS

A. Exposure levels

The internal doses that the test animals received could not be calculated from the information provided.

B. Mortality

One male cat receiving 0.6 mL/application was found dead on day 36. From p. 24 of MRID 44850103: "No notable clinical signs were ever observed during the study for this animal, no gross abnormal findings were observed at necropsy. No cause of death was determined for this animal... Since this mortality occurred at the end of study in an animal from the low dose group that had not shown any abnormal clinical sign during the duration of the study, it was not considered to be treatment-related."

C. Clinical signs

No notable clinical signs were observed in any treated or control animal.

D. Bodyweight and weight gain

No significant differences in group body weight gains occurred in any test group.

E. Food consumption

No significant difference in food consumption occurred between treated and control animals.

F. Electrocardiography

The mean values are presented by sex and dosage group (Tables 5-10, pp. 37-42 of MRID 44850103). There were no significant differences between treated and control groups.

G. Ophthalmology

There were sporadic occurrences of focal corneal opacification, but in at least some instances this was transient. This was not dose-related.

H. Hematology

No treatment-related changes were recorded in any of the hematological parameters examined at the end of each treatment period (day 4 and day 22) when compared to pretreatment and control values.

I. Clinical chemistry

Moderate to markedly lower total cholesterol values (-24 to -52%) occurred at all sampling times in one male cat treated with 1.2 mL/treatment and in one female treated with 1.8 mL/treatment. These changes were not considered to be

treatment-related because no trend was observed in females at the intermediate dose or in males at the highest dose. No other significant differences were observed in clinical chemistry between treatment and control or pre-treatment values.

J. Necropsy findings

Necropsy was performed all animals on day 39. No necropsy findings were noted in the one male that died during the study. One female from the low-dose group showed a thickened urinary bladder with reddish foci. This was not considered to be treatment-related because similar effects were not seen in any of the animals in the higher dose groups.

IV. DISCUSSION

In a companion animal safety study (MRID 44850103), a formulation containing 10% pyriproxyfen as sole active was topically applied to groups of three male and three female 3-month old kittens for two periods of three consecutive days (days 1, 2, and 3 and then days 19, 20 and 21) at 1X (0.6 mL/treatment), 2X (1.2 mL/treatment), and 3X (1.8 mL/treatment) label-indicated dosing levels/day (3X, 6X and 9X over each 3-day dosing period). Controls were treated with 1.8 mL of excipient. While the number of animals/sex/dosage is below the six specified in OPPTS 870.7200, the study was initiated on July 1, 1997, and completed on February 2, 1998, before the publication (August 1998) of the OPPTS Harmonized Test Guidelines. Test animals were observed for toxicologic effects once per hour for 4 hr after each application and daily during the entire 36-day study. Food consumption was monitored daily. Body weights were recorded twice before treatment began and once a week thereafter. Blood samples for hematological and biochemical analyses were taken before treatment began and 24 hr after the last dose of each treatment period. Blood samples for cholesterol analysis were taken on days 10 and 28. Urinalysis, as well as electrocardiographic and ophthalmological examinations were performed before treatment and on days 18 and 36. All animals were sacrificed at the end of the study, with post-mortem macroscopic examinations conducted on all. The skin application site was examined microscopically. Sacrifice and necropsy are not required by the Companion Animal Safety Study Guidelines, although as previously noted this study was conducted before the August 1998 publication of the OPPTS Harmonized Guidelines.

Although one animal died during the study, this death was not considered to be treatment-related. Microscopic examination did not reveal any treatment-related effects on the skin. Upon necropsy one female from the low-dose group showed a thickened urinary bladder with reddish foci, not considered to be treatment-related because similar effects were not seen in any of the animals in the higher dose groups. Food consumption and body weights were not affected by treatment. Moderate to markedly lower total cholesterol values (-24 to -52%) occurred at all sampling times in one male cat in the 1.2 mL/treatment group and in one female in the 1.8 mL/treatment group. These changes were not considered to be treatment-related because no trend was observed in females at the intermediate dose or in males at the highest dose. There were no other changes in clinical chemistry or hematology that could be attributed to treatment. Electrocardiographic and ophthalmological examinations did not reveal any abnormalities in the treated animals.

Although no single daily application of pyriproxyfen exceeded a 3X dosing level, a cumulative 6X exposure was achieved by the second day and a cumulative 9X exposure by the third day. The product was applied again to the same test animals 15-17 days after the first treatment with no adverse effects. Product labeling and

application instructions state that it should be reapplied every three months. The label includes the statement: "Do not treat kittens less than one month." However, the kittens used in this study were three months old, and the statement should therefore be revised to stated: "Do not treat kittens less than three months." While there are questions as to whether or not the dsoage schedule really demonstrated a 5X margin of safety, it is noted that the proposed product is packaged in pipettes, and would be dispensed in measured amounts of 0.6 g/application. As long as this product is packaged in this manner, this study is Acceptable to support the proposed use on cats over the age of 3 months. It is also noted that the available data indicate that the active ingredient in this formulation (Pyriproxyfen) has been demonstrated to have low toxicity to mammalian species.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D257141
2. **PC CODE:** 129032 NYLAR (also known as PYRIPROXYFEN)
3. **CURRENT DATE:** June 15, 1999
4. **TEST MATERIAL:** Pyriproxyfen 10% Spot On For Cats, a colorless liquid containing 10% Pyriproxyfen (Nylar)

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety/3-Month Old Kittens/Centre International de Toxicologie (France)/636.29/FEB-2-1998	44850103	No dose-related mortality (one incidental death) toxicological signs, or indications of changes in clinical chemistry or hematology parameters, or indications of dermal effects, were noted in groups of 3M, 3F 3-month old kittens following dermal application (to back of neck) at 1X (0.6 mL/treatment), 2X (1.2 mL/treatment), or 3X (1.8 mL/treatment) label recommended doses. Controls were dosed with vehicle only at 1.8 mL/treatment. As the formulation was applied for two periods of 3 consecutive days (days 1,2,3 & then days 19,20, 21) doses applied were actually 3X, 6X and 9X label recommended doses over each 3-day period.	-	A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self-Validated**

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of Prevention, Pesticides
and
Toxic Substances

October 19, 1999

MEMORANDUM

SUBJECT: Secondary Review of DERs for Companion Animal Safety Studies

DP Barcode: D259943
Submission: S563686
PC Code: 129032
MRID: 44850103

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Reregistration Branch I, Health Effects Division (7509C)
Virginia A Dobozy 10/19/99

TO: John Redden, Branch Senior Scientist
Technical Review Branch, Registration Division (7505C)

Action Requested: Provide secondary review of DER for MRID

Recommendation: The HED Companion Animal Safety Committee met on October 13, 1999. See attached Conclusions of the meeting.

Memorandum of Companion Animal Safety Committee Meeting - October 13, 1999

The following DER prepared by the Technical Review Branch, RD, was discussed.

1) DP Barcode: D259943

Product: 2382-RTG Pyriproxyfen 10% Spot On for Cats

Study Title: Tolerance Study by Cutaneous Application (Spot On) in Cats

The following is the executive summary for the study in MRID 44850103:

In a companion animal safety study (MRID 44850103), a formulation containing 10% Pyriproxyfen as sole active was topically applied to groups of three male and three female 3-month old kittens for two periods of three consecutive days (days 1, 2, and 3 and then days 19, 20 and 21) at 1X (0.6 mL/treatment), 2X (1.2 mL/treatment), and 3X (1.8 mL/treatment) label-indicated dosing levels/day (3X, 6X and 9X over each 3-day dosing period). Controls were treated with 1.8 mL of excipient. Test animals were observed for toxicologic effects once per hour for 4 hr after each application and daily during the entire 36-day study. Food consumption was monitored daily. Body weights were recorded twice before treatment began and once a week thereafter. Blood samples for hematological and biochemical analyses were taken before treatment began and 24 hr after the last dose of each treatment period. Blood samples for cholesterol analysis were taken on days 10 and 28. Urinalysis, as well as electrocardiographic and ophthalmological examinations were performed before treatment and on days 18 and 36. Post-mortem macroscopic examinations were conducted on all test animals. The skin application site was examined microscopically.

Although one animal died during the study, this death was not considered to be treatment-related. Microscopic examination did not reveal any treatment-related effects on the skin. Upon necropsy one female from the low-dose group showed a thickened urinary bladder with reddish foci, not considered to be treatment-related because similar effects were not seen in any of the animals in the higher dose groups. Food consumption and body weights were not affected by treatment. Moderate to markedly lower total cholesterol values (-24 to -52%) occurred at all sampling times in one male cat in the 1.2 mL/treatment group and in one female in the 1.8 mL/treatment group. These changes were not considered to be treatment-related because no trend was observed in females at the intermediate dose or in males at the highest dose. There were no other changes in clinical chemistry or hematology that could be attributed to treatment. Electrocardiographic and ophthalmological examinations did not reveal any abnormalities in the treated animals.

This study generally followed the pertinent guidelines for a companion animal safety study (OPPTS 870.7200). Although no single daily application of pyriproxyfen exceeded a 3X dosing level, a 6X exposure was achieved by the second day and a 9X exposure by the third day. The product was applied again to the same test animals 15-17 days after the first treatment with no adverse effects. Product labeling and application instructions state that it should be reapplied

every three months. The label includes the statement: "Do not treat kittens less than one month." However, the kittens used in this study were three months old, and the statement should therefore be revised to stated: "Do not treat kittens less than three months." Otherwise, the required 5X margin of safety has been demonstrated and the study is Acceptable to support the proposed use on cats.

Discussion and Conclusions from CAS Meeting

1. The waiver of an acute inhalation study, which is discussed in the Memorandum accompanying the DER, is considered appropriate.

2. The Committee concluded that dosing at 1x, 2x and 3x on Days 1, 2 and 3 and again on Days 19, 20 and 21 does not constitute 3x, 6x and 9x the recommended level of treatment. The treatment levels should be considered 1, 2 and 3x the recommended dose. Therefore, the study did not demonstrate a 5x margin of safety.

3. The Executive Summary should be more explicit in noting that all animals were sacrificed and necropsied at the end of the study. Sacrifice and necropsy are not required by the Companion Animal Safety Study Guidelines.

4. Although not discussed at the CAS meeting, it should be noted that there were only three kittens/sex/group in the study. The Companion Animal Safety Study Guidelines require six animals/sex/group.

5. The CAS Committee was polled via email after the meeting about the acceptability of the study. The Committee concluded that, given dosing at less than 5x the label recommended treatment and the use of only three animals/sex/group, RD should reconsider whether this study should be classified as acceptable.

Prepared by Virginia A. Dobozy, V.M.D., M.P.H.
Reregistration Branch 1, HED (7509C)

JUN 21 1999

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs

VIRBAC INC.
P.O. BOX 162059
FORT WORTH, TX 76161

Report of Analysis for Compliance with PR Notice 86-5

Thank you for your transmittal of 04/21/99. Our staff has completed a preliminary analysis of the material. The results are provided as follows:

Your submittal was found to be in full compliance with the standards for submission of data contained in PR Notice 86-5. A copy of your bibliography is enclosed, annotated with Master Record ID's (MRIDs) assigned to each document submitted. Please use these numbers in all future references to these documents. Thank you for your cooperation. If you have any questions concerning this data submission, please raise them with the cognizant Product Manager, to whom the data have been released.